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Psoralen and Long Wavelength Ultraviolet Radiation as an Adjuvant Therapy for Prevention of Intimal Hyperplasia and Constrictive Remodeling After Balloon Dilation: A Study in the Rabbit Iliac Artery

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Background and Objective: **Restenosis after balloon angioplasty is the summated effect of intimal hyperplasia and arterial shrinkage, both caused by hyperproliferation. In the present study, the potential of a photochemotherapeutic modality (Psoralen + UVA: PUVA) for the prevention of angioplasty induced proliferation was explored.**
Study Design/Materials and Methods: **In rabbit iliac arteries, balloon dilation followed by PUVA-therapy (H = 1 J/cm^2) was performed (n = 15). Contralateral arteries served as control. After 2 and 28 days of survival, the contribution of intimal hyperplasia and remodeling to lumen loss was determined by means of angiography and histological analysis.**
Results: **After 2 days, large parts of the media had become acellular, while proliferation was occurring predominantly in the adventitia in both groups. After 28 days, late loss, arterial shrinkage, but not intimal hyperplasia were larger in the PUVA group ($P < 0.05$).**
Conclusion: **PUVA-therapy did not prevent intimal hyperplasia following balloon dilation but enhanced luminal narrowing by augmented constrictive remodeling. Lasers Surg. Med. 23:281–290, 1998.** **© 1998 Wiley-Liss, Inc.**

Key words: angioplasty; cardiovascular disease; PUVA; photochemotherapy; restenosis

INTRODUCTION

Although successful initially, the long-term benefit of percutaneous transluminal coronary angioplasty (PTCA) is restricted by restenosis within 6 months, requiring follow-up intervention such as angioplasty or bypass surgery in about 30% of the cases [1]. This restenosis phenomenon can be viewed as a reaction to damage in the blood vessel wall that is induced by the angioplasty procedure. Proliferation of and extracellular matrix deposition by dedifferentiated vascular smooth muscle cells (SMCs) originating from the media lead to intimal hyperplasia [1,2]. However, abun-

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dant cell proliferation in the adventitia [3,4], culminating into arterial shrinkage or constrictive remodeling, has recently been recognized as another mechanism contributing to restenosis [1,3,5]. Stenting is suitable for the prevention of shrinkage [1,6]. Unfortunately, this procedure has the disadvantage of augmenting the intimal hyperplasia response to interventional injury that may result in in-stent restenosis [1,6]. Thus, local inhibition of cell proliferation (being the major determinant of the reaction to angioplasty induced damage) is an attractive strategy to increase the long-term therapeutic effectiveness of mechanical intervention procedures [7].

Psoralens (or furocoumarins, a group of photosensitizers that have proven their merit in dermatological practice for some decades [8,9]), show potential for the inhibition of proliferation of SMCs in vitro, a process that is thought to be mediated by photochemotherapy (PCT) [10,11]. Upon photo-activation with UVA radiation, psoralens have been shown to inhibit the proliferation of SMCs in various animal models [12-14]. In addition, PUVA-therapy (an acronym for **P**soralen + **UVA**) reduced morphometric and angiographic stenosis after 14 days of survival [14]. Yet, the long-term effectiveness of PUVA angiographic outcome has not been investigated thus far. This study addresses the potential of PUVA-therapy, at a radiant exposure of 1 J/cm2, for the prevention of intimal hyperplasia and arterial shrinkage in a rabbit vascular injury model. Previous experiments in our laboratory have indicated that this radiant exposure value sufficiently activates the photosensitizer while minimizing the amount of additional damage to the vessel wall (unpublished data). In this study, we compared angiographic and histologic results at 2 and 28 days of survival after experimentally induced arterial wall injury (PTA balloon dilation) and photosensitizer administration with (PUVA group) or without (PTA group) UVA irradiation.

MATERIALS AND METHODS

Experimentally Induced Injury

Fifteen healthy ELCO rabbits were used in this study. Animal care conformed to the "Position of the American Heart Association on Research Animal Use" and to the guidelines of the Faculty Commission on Animal Experiments of the Utrecht University. The rabbits were housed in groups and received a normal diet throughout the course of the experiment.

Before each procedure, the rabbit was anesthetized as described previously [15] and a 5 F sheath was inserted into the right carotid artery with its tip in the descending aorta. Rabbits received a dose of 100 IU Heparin/kg. Angiography of the iliac arteries was performed by a C-arm (Philips, Best, The Netherlands) before, during and after the procedure and at follow-up. Angiograms were digitized and stored.

Subsequently, a modified 3.0 mm diameter, 25 mm length PTA balloon catheter (SciMed Life Systems, Minneapolis, MN) was advanced under fluoroscopy into the iliac artery. The PTA balloon was inflated to a pressure of 10 bar with deionized water and left at its position for approximately 9 min (depending on the power delivered by the light source) while PUVA-therapy (see below) was performed (treatment group: PUVA). The contralateral artery received the same treatment without activation of the photosensitizer (control group: PTA). The PUVA-therapy was randomly allocated to the left or right iliac artery. The angiographic images were digitized and stored in order to obtain a mark for the exact position of the balloon and eventual UV light delivery. After all procedures, the PTA balloon was deflated and the balloon catheter (with UV-light application device) and sheath were withdrawn, followed by ligation of the carotid artery.

PUVA-Therapy

Thirty milligrams of eight-methoxypsoralen (8-MOP) (Sigma Chemical Co., St. Louis, MO) was dissolved in 3 ml dimethyl sulphoxide (DMSO) (BDH Laboratory Supplies, Poole, England) (1% w/v). This solution was further diluted in saline at 40°C. The dilution factor (1:40 for bolus infusion and 1:100 for i.v. infusion for a 5 kg rabbit) depended on the weight of the rabbit. It was chosen to result in the same amount of 8-MOP to be received by each rabbit per unit body weight, while the infusion rate and the volume of solution being administered were kept constant for all rabbits. Rabbits received a 20 ml bolus of 8-MOP solution (1 mg/kg body weight) i.v. at a rate of 4 ml/min. After 5 min, 8-MOP solution (1 mg/kg body weight /hour) i.v. infusion was started at a rate of 1 ml/min. After a minimal infusion time of 10 min (to allow for proper diffusion of 8-MOP into the arterial wall tissue; Keith March, Krannert Institute of Cardiology, Indiana University; personal communication, February 1996), the balloon angioplasty procedure was started.

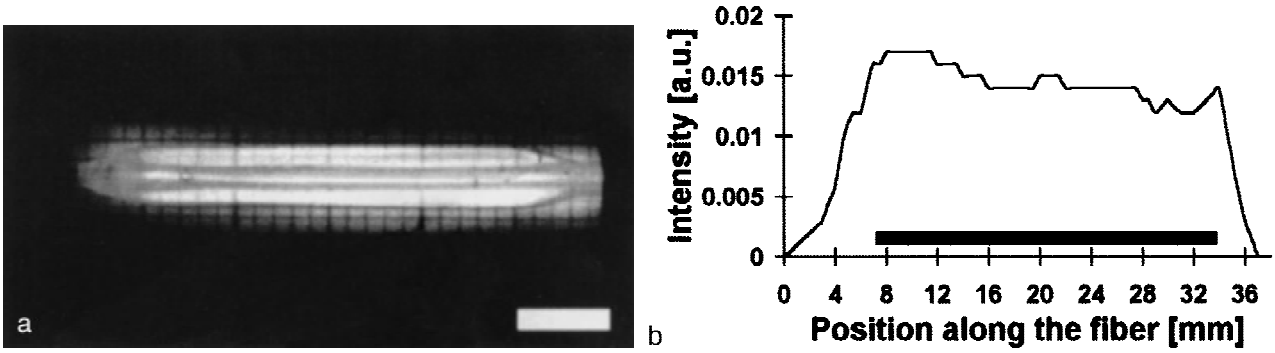

Fig. 1. **a:** Photomicrograph of the diffusing *Lightstic,* inserted into the guide wire lumen of the modified balloon catheter (3.0 mm diameter). Note the guide wire tip at the distal end (left side of the picture) of the balloon catheter. Bar: 500 μm. **b:** Intensity profile of the diffusing *Lightstic* at the surface of the inflated PTA balloon (distance from central axis is 1.5 mm). The black bar represents the position of the balloon.

Irradiation was performed with an Argon-ion laser (Innova 90, Coherent laser products, Palo Alto, CA) at 355 and 365 nm wavelengths in continuous mode. A diffusing fiber rod (length 24.0 mm, diameter 300 μm) with a scattering surface (*Lightstic,* Rare Earth Medical, Inc., West Yarmouth, MA) was used for the application of UV-light to the vessel wall (Fig. 1a). The *Lightstic* was inserted into the guide wire lumen of a modified PTA balloon catheter. The intensity profile of the *Lightstic* (Fig. 1b) was assessed to be homogenous with an epoxy spherical probe (diameter = 250 μm) connected to a photodiode.

The modification of the balloon catheter comprised the removal of the angiographic marker from the middle part of the balloon and the sealed application of a segment of guide wire tip into the distal guide wire lumen (Fig. 1a). By this modification, no shadowing of the UV-light on the artery wall as a consequence of the mid balloon angiographic marker was encountered, while the sealing of the guide wire tip into its lumen prevented the inflow of blood into the guide wire lumen, which houses the UV-light application device. Before and after each experiment, the output power of the *Lightstic* encased by the PTA balloon was measured with an integrating sphere. After positioning and inflation of the balloon, UV-light was applied to the vessel wall at a radiant exposure $H = 1\ J/cm^2$. Because of a dilation ratio>1 (see below), the blood was squirted away by the inflated balloon and direct irradiation of the vascular wall was achieved. The contralateral artery received no radiation. After both angioplasty procedures had been completed, a blood sample was taken (approximately 40 min after the start of 8-MOP infusion) and 8-MOP infusion was stopped. Subsequently, the 8-MOP serum concentration was assessed.

The blood samples were spun for 10 min at 1,500 rpm and the supernatant was removed. For each blood sample, 1 ml supernatant was purified, along with 100 μl 5-methoxypsoralen (5-MOP) internal standard, by liquid phase extraction in 5 ml heptane/ dichlorinemethane 4/1. Five-MOP was chosen as an internal standard because it is a structural isomer of 8-MOP and its peak on the chromatogram does not coincide with the 8-MOP peak (retention times: 8-MOP = 2.6 min, 5-MOP = 3.7 min). After shaking for 5 min (1,400 rpm), the samples were centrifuged for 10 min at 3,000 rpm. The supernatant was removed and vaporized at 40°C. After cooling down, the residue was dissolved in 50 μl eluting solvent (methanol/ water 65/35) and 25 μl aliquots were injected into the 125 × 4 mm column (Lichrocart 100 RP-18, Merck) of the HPLC system (Hewlett Packard, Amstelveen, The Netherlands) at 25°C and analyzed at 247 nm. The 8-MOP concentration was determined with a calibration curve. For analysis, late loss (see below) was plotted as a function of the serum sensitizer concentration.

Angiographic Evaluation

The angiographic diameters of the arteries were measured using a semi-automated program with digital calipers. The quantitative edge detection algorithm is applied on the digitized gray value of a proposed line perpendicular to the center axis of the lumen. The gray value distribution along the perpendicular line has its maximum outside the lumen and its minimum in the middle of the lumen. The edge of the lumen was defined by the pixel with a gray value equal to the average

of the maximum and minimum gray values. The diameter of the arterial segment was calculated with this full-width-half-maximum distance. The standard error of the y-estimate was assessed to be 0.087 mm by means of calibrated phantoms with diameters varying from 0.40 to 4.00 mm filled with contrast agent, while $R^2 = 0.997$. In each artery, lumen diameters were measured at seven positions: one proximal and one distal reference site and five sites equidistantly spaced (6 mm) within the balloon dilated segment. To use equal positions at different time points (pre, during, and post procedure and at follow-up), the seven positions were documented relative to an anatomic landmark. Angiographic measurements were calibrated using a radiopaque ruler.

The mean luminal diameter at the lesion position was determined by averaging the separate values for the five sites within one balloon dilated segment. Acute gain was defined as the difference between post and pre procedure mean lumen diameters. Late loss was defined as the difference between post procedure and follow-up mean lumen diameters. Total loss was defined as the difference between pre procedure and follow-up mean lumen diameters. The dilation ratio was defined as the angiographic pre dilation lumen diameter divided by the balloon diameter. The balloon diameter was measured after the procedure.

Sacrifice and Histologic Processing

Two and twenty-eight days after PUVA-therapy, the rabbits were sacrificed by an overdose of sodium pentobarbital (60 mg/ml i.v.). A mid-abdominal incision was made and the descending aorta and inferior vena cava were ligated cranially. The arteries were saline perfused in situ at 60 mm Hg.

For the 28 day survival group, the saline perfused arteries were pressure perfused by a mixture of contrast medium and Agar at a temperature of 50°C, as described before [16]. This mixture, which congealed in the arteries, prevented collapse of the vessels during fixation. The arteries were then fixed in situ and peri-adventitially with formalin 4%. For the 2 days survival group, the arteries were fixed in formalin 4% without pressure perfusion. After more than 24 h of fixation, the arteries were divided into 6 mm segments.

All segments were dehydrated and embedded in paraffin. The segments were cut in duplicate 5 micron cross sections and stained with Hematoxylin and Eosin (H&E) and Elastin von Gieson (EvG). Proliferation was detected with the monoclonal MIB-1 antibody, which reacted to the human nuclear antigen Ki-67 (2 ng/ml, Immunotech, Westbrook, ME). Sections were stained according to the indirect alkaline phosphatase method. Slides were preincubated for 30 min with 10% normal horse serum and, after decantation, again for 60 min with the primary antibody. The slides were then rinsed in PBS, incubated for 45 min with a secondary antibody, rinsed in PBS, and incubated for 60 min with alkaline phosphatase conjugated streptavidin (Dakopatts, Glostrup, Denmark). Horse biotinylated anti-mouse (Vector Laboratories, Burlingame, CA) was used as a secondary antibody. Alkaline phosphatase was visualized with naphthol (0.05% w/v, 30 min)-New Fuchsin. For negative controls, the primary antibody was omitted.

Light Microscopic Evaluation

In the 2 day survival group, arterial wall damage was scored quantitatively as the percentage of the total medial area that was devoid of SMCs (percentage medial acellularity) in the Hematoxylin and Eosin stained sections at locations similar to the angiographic measuring positions. One proximal and one distal reference segment and five cross-sections in the balloon dilated part of the artery were analyzed. In the same survival group, proliferation was scored by the number of MIB-1 positive nuclei of non-inflammatory cells per unit of circumference of the lumen [/mm].

In the 28 day survival group, lumen and media bounded area were assessed quantitatively in mm^2 in Elastin von Gieson stained cross-sections with a digital image processing system (AnalySIS, Münster, Germany), also at locations similar to the angiographic measuring positions. From these areas, the mean intimal hyperplasia thickness was calculated in each cross section, assuming circular anatomy. The values for each cross section from a single artery were averaged to yield the mean thickness for each artery.

Luminal narrowing after interventional injury is the result of both intimal hyperplasia formation and shrinkage of the artery. Shrinkage of the artery was assessed by subtracting the average thickness of the intima on histology from the average luminal narrowing on serial angiography (late loss or total loss) in each balloon dilated segment, as described previously [3].

Statistical Analysis

Angiographic and histologic data are presented as mean ± standard error of the mean

TABLE 1. Angiographic Diameters at Different Time Points After Intervention (PTA or PUVA)

Survival	Treatment	Pre angiographic diameter (mm)	Post angiographic diameter (mm)	Follow-up angiographic diameter (mm)
2 days	PTA	1.96 ± 0.11	2.33 ± 0.09	2.21 ± 0.14
	PUVA	1.91 ± 0.11*	2.36 ± 0.10	2.30 ± 0.11
28 days	PTA	2.16 ± 0.06	2.38 ± 0.07	1.96 ± 0.09**
	PUVA	2.14 ± 0.06	2.51 ± 0.07	1.71 ± 0.12

*$P < 0.05$ pre vs. post angiographic diameter.
**$P < 0.05$ PTA vs. PUVA.

(S.E.M.). Differences in mean angiographic lumen diameters, percentage medial acellularity, number of proliferating non-inflammatory cells, and intimal hyperplasia, as well as the percentage medial acellularity and number of proliferating cells per position, between the PTA group and the PUVA group were assessed by a two-tailed paired t-test. Differences within groups, with respect to percentage medial acellularity and number of proliferating cells per position, were assessed by a one-way ANOVA with Bonferonni test. Function coefficients and correlations were determined by linear regression analysis (least squares method). R-squared value significances were determined with the F-statistic. Differences were considered significant at a level of $P < 0.05$.

RESULTS

Two Days Survival

Angiography. The mean angiographic luminal diameters of the lesion before, immediately after and at 2 days follow-up after balloon dilation with or without 8-MOP photo-activation are presented in Table 1 (n = 5 for both groups). Dilation ratios did not differ between both groups (PTA vs. PUVA: 1.53 ± 0.08 vs. 1.57 ± 0.08; $P = 0.42$). No statistically significant differences in luminal diameters were observed between both groups. However, a significant difference between pre and post angiographic diameter was observed within the PUVA group.

Arterial wall damage. Two days after balloon dilation with or without PUVA-therapy, acellularity in large parts of the media was observed in both groups (n = 5) (Fig. 2a,b). No dissections and only marginal leukocyte infiltration were witnessed. No differences between the two groups in percentage medial acellularity for the seven measuring positions, as well as for the mean of the lesion and reference segment positions in each artery, were found. Acellularity was not as excessive towards the edges as in the middle part of the lesion ($P<0.001$ for both groups) (Fig. 3).

Histochemistry. Proliferation was assessed at 2 days following intervention. In both groups, MIB positive nuclei could be detected in the artery wall, predominantly in the adventitia. Proliferation in the media was minor and confined to the edges of the lesion segment, while adventitial proliferation was especially notable in the mid-lesion segments (Fig. 4a,b). As is the case for the percentage medial acellularity, no difference in the number of non-inflammatory proliferating cells per unit of circumference between the two groups for each of the seven measuring positions was encountered, both in the media and the adventitia. This is also true for the mean of lesion and reference segments.

A significant difference in the mean number of non-inflammatory proliferating cells between the lesion and reference segments of each artery was found in the adventitia ($P<0.001$ for both groups) but not in the media. In addition, correlations between the percentage medial acellularity and the number of proliferating cells were determined. No correlation was found between the number of proliferating cells in the media and medial acellularity. However, significant positive correlations were found between the number of adventitial proliferating cells and medial acellularity percentage for the different measuring positions, with $R^2 = 0.32$ ($P = 0.0003$) for the PTA group and $R^2 = 0.42$ ($P < 0.0001$) for the PUVA group.

Twenty-Eight Days Survival

Angiography. The mean angiographic diameter changes of the lesions at the different time points after balloon dilation with or without PUVA-therapy are presented in Figure 5 (n = 10 for both groups). Dilation ratios did not differ (PTA vs. PUVA: 1.38 ± 0.04 vs. 1.39 ± 0.04). Statistically significant differences in acute gain, late

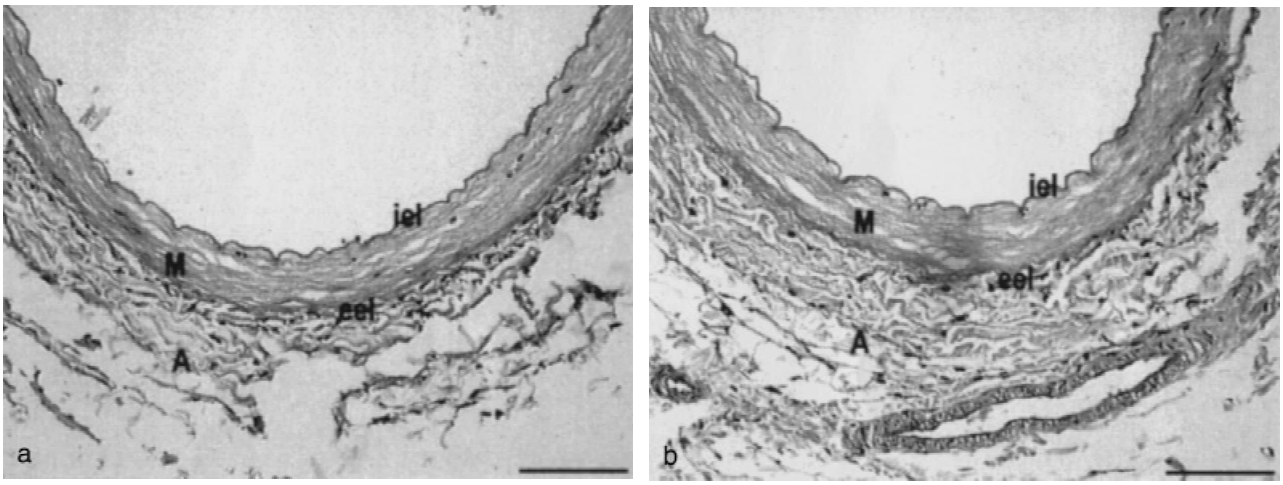

Fig. 2. Photomicrograph of a cross section (H&E) of a rabbit iliac artery two days after intervention: PTA (**a**) and PUVA (**b**). Note the almost complete acellularity of the media (magnification ×100). M, media; A, adventitia; iel, internal elastic lamina; eel, external elastic lamina. Bar: 100 μm.

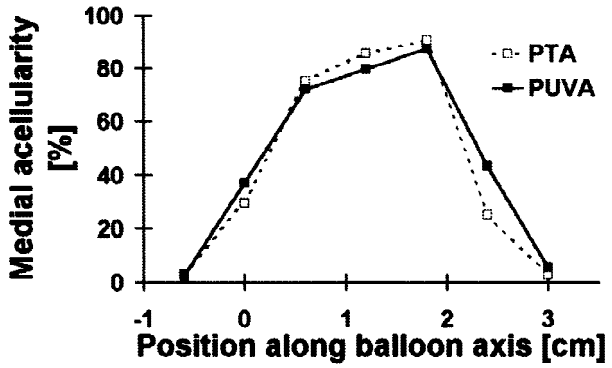

Fig. 3. Percentage medial acellularity two days after intervention (PTA or PUVA) as a function of the position along the balloon axis. The balloon is positioned from 0 (proximal) to 2.4 (distal) cm.

loss and total loss were observed between both groups, with a larger acute gain and a larger late as well as total loss in the PUVA group ($P < 0.05$) (Fig. 5). Angiographic diameters at follow-up were significantly greater in the PTA group ($P = 0.035$) (Table 1). In both groups, lumen boundaries were smooth and occlusions were absent.

Histomorphometry. Histological analysis revealed that at least part of the luminal narrowing after intervention can be ascribed to intimal hyperplasia. Lesion segments were characterized by a regular intimal hyperplasia (Fig. 6a,b) with smooth muscle cells oriented longitudinally near the internal elastic lamina and circumferentially near the lumen. However, no significant difference in mean intimal hyperplasia thickness between the two groups could be detected (2*IH: PTA vs. PUVA: 0.08 ± 0.05 mm vs. 0.11 ± 0.06 mm; $P = 0.30$) (Fig. 5). Reference segments were devoid of intimal hyperplasia.

Remodeling. The angiographic diameter loss could only partly be explained by the amount of intimal hyperplasia (Fig. 5). This indicates the importance of arterial wall shrinkage (constrictive remodeling) on luminal narrowing following arterial wall injury. The larger late and total loss in the PUVA group is explained by the greater amount of arterial wall shrinkage compared to the PTA group (late loss shrinkage PTA vs. PUVA: 0.33 ± 0.08 mm vs. 0.70 ± 0.12 mm, $P = 0.018$; total loss shrinkage PTA vs. PUVA: 0.11 ± 0.07 mm vs. 0.32 ± 0.11 mm, $P = 0.045$).

Eight-MOP serum levels. One of the serum samples was discarded because of its extremely high concentration (C = 7695 ng/ml). The mean 8-MOP serum level of the remaining samples (n = 9) was 162 ± 25 ng/ml. Correlations between 8-MOP serum concentration (C) and angiographic late loss (LL) were weak and not significant, although the correlation was higher and tended towards significance in the PUVA group (PTA: LL = 0.001*C + 0.2482, $R^2 = 0.0821$, $P = 0.45$; PUVA: LL = 0.0033*C + 0.2625, $R^2 = 0.319$, $P = 0.11$).

DISCUSSION

In this study, we investigated the potential of PUVA-therapy for the prevention of intimal hyperplasia formation and arterial shrinkage after balloon dilation of the rabbit iliac artery. The principal finding of our study is that, although no

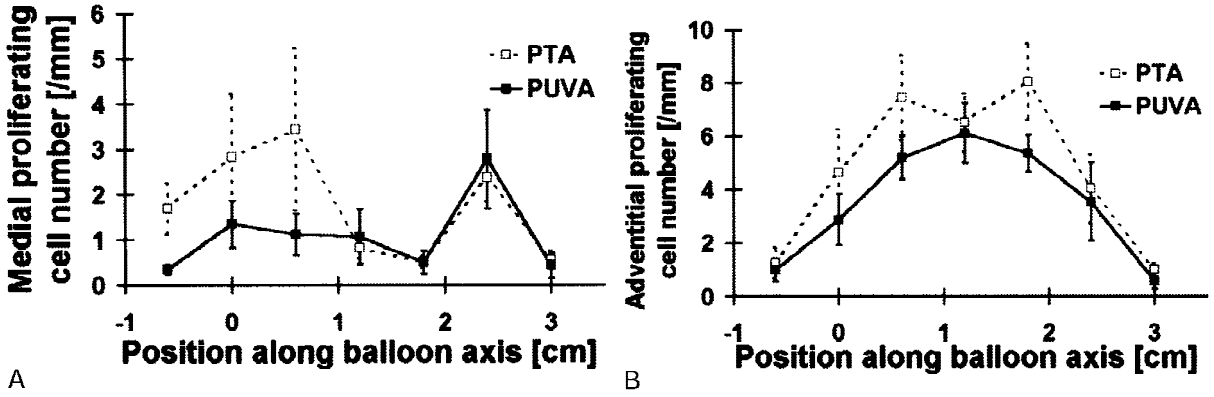

Fig. 4. Number of proliferating smooth muscle cells two days after intervention (PTA or PUVA) as a function of the position along the balloon axis. **A:** media; **B:** adventitia (note the difference in scale). The balloon is positioned from 0 (proximal) to 2.4 (distal) cm.

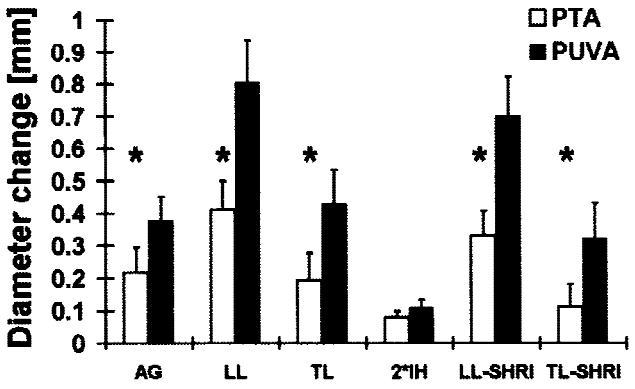

Fig. 5. Changes in angiographic diameter of normal rabbit iliac arteries after intervention: acute gain (AG), late loss (LL) and total loss (TL) at 28 days. The contributions of intimal hyperplasia (2*IH) and arterial shrinkage (LL/TL-SHRI) to the late/total loss in both groups are also depicted. *P<0.05 between groups.

induction of additional damage to the arterial wall was encountered, PUVA-therapy enhanced luminal narrowing due to augmented arterial shrinkage under the specific conditions applied in our study.

Short-Term Effects

After 2 days of survival, large parts of the media of the balloon dilated arterial segment had become acellular with hardly any infiltration of leukocytes. The amount as well as the distribution of the medial acellularity percentage (with a distinct maximum in the middle of the dilated segment) was similar in both groups (Fig. 3). This damage profile can be attributed to the force distribution within the arterial wall due to balloon dilation. The light distribution of the *Lightstic* was homogeneous (Fig. 1b). Consequently, if the UVA light would have induced additional damage, an increased medial acellularity would have been observed at the edges of the dilated segment (positions 0.0 and 2.4 cm in Fig. 3).

Furthermore, no differences in mean proliferation of cells, as well as for the different measuring positions, were witnessed between both groups after two days of survival. Both in the media and the adventitia, MIB positive nuclei could be identified, albeit only marginal in the media. In the media, these positive nuclei were located at the edges of the balloon dilated arterial segment. Conversely, in the adventitia positive nuclei were concentrated in the central part of the injured segment. The number of proliferating cells in the adventitia correlated with the medial acellularity percentage for the different measuring positions. We hypothesize that either the induced medial damage or its mechanical origin is the cause of the proliferative response in the adventitia. Therefore, it appears that PUVA-therapy is unable to prevent proliferation of smooth muscle cells in the media and adventitia at 2 days after balloon dilation in the rabbit at the dose tested.

The observations with regard to acellularity of the media are partly in agreement with previous studies. Arterial wall injury followed by photo-activation of different sensitizers gives rise to an acellular media, after at least seven days of survival in rats and the pig [17–20]. However, in our study, arterial wall injury without photo-activation of the sensitizer also gives rise to a large amount of acellularity of the media at two days of survival, which is in contrast with observations in the rabbit after a same survival period [21]. It should be noted that a different injury model was used in this study (Fogarty denuda-

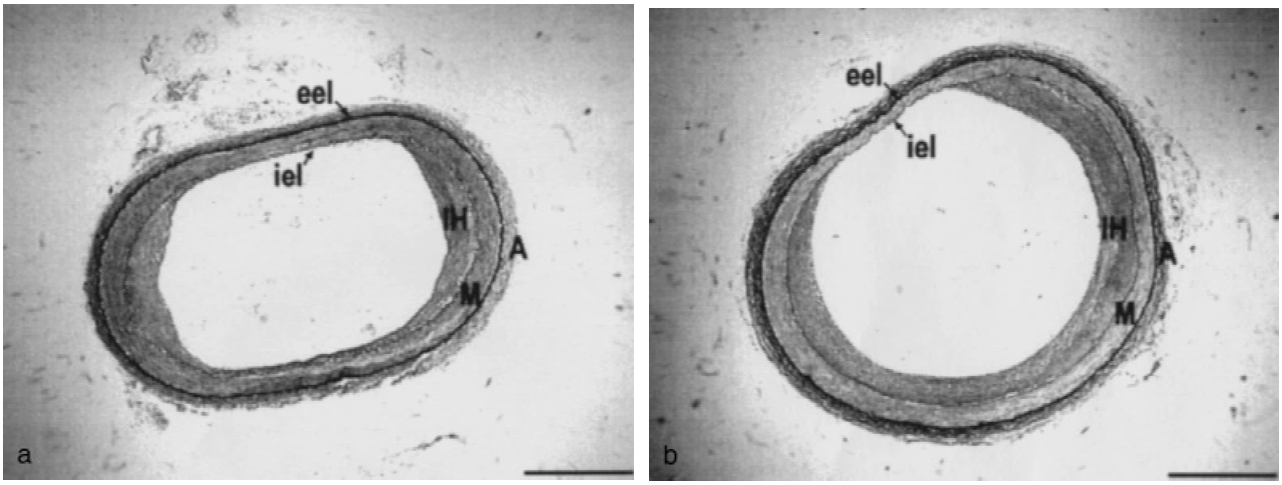

Fig. 6. Photomicrograph of a cross section (EvG) of a rabbit iliac artery 28 days after intervention: PTA (**a**) and PUVA (**b**). Note the intimal hyperplasia which spans almost the entire circumference of the blood vessel (magnification ×20). M, media; A, adventitia; iel, internal elastic lamina; eel, external elastic lamina; IH, intimal hyperplasia. Bar: 500 μm.

tion) in comparison with our injury model (balloon dilation at a ratio of 1.53). In addition, the working mechanism in our study is the photoactivation of 8-MOP, which presumably covalently interacts with the DNA of the target cells, giving rise to a cell cycle block and inhibition of proliferation, although other mechanisms have been proposed [22]. In unpublished pilot experiments, we have demonstrated that covalent interactions between 8-MOP and the target cell DNA could be detected after UVA irradiation in a dose-dependent manner; this was accomplished with the 8G1 antibody, kindly supplied by Regina M. Santella [23]. The mechanism of covalent interaction, however, is different from the singlet oxygen mediated photodynamic therapy (PDT) mechanism, that is aimed at the destruction of the target cells, as used in the above mentioned studies [17–20].

Furthermore, in preliminary reports of other studies in the pig [13] and in the atherosclerotic rabbit [12], PUVA-therapy was found to significantly inhibit SMC proliferation after 2 and 5 days of survival, respectively. These differences with regard to our study may be explained by the different injury models, which did not lead to an acellular media, that were used. The injury procedure in our model, however, has led to the loss of the possible medial substrate for PUVA-therapy.

Long-Term Effects

In both groups, intimal hyperplasia and arterial shrinkage contributed to the angiographically observed late loss in lumen diameter, which is in agreement with previous observations within our laboratory [3]. Although no significant differences were found between the two groups after 2 days, after 28 days arterial shrinkage was larger in the PUVA group (Fig. 5). This resulted in a smaller angiographically measured lumen (Table 1) and, consequently, a greater late and total loss compared to the PTA group (Fig. 5). Thus, PUVA-therapy actually augmented luminal narrowing after balloon dilation. The mechanism underlying this process remains to be elucidated but may be due to the direct interaction of the UV light itself or in combination with 8-MOP with the arterial wall.

It is unlikely that a larger UVA dose would have had a more beneficial effect on luminal narrowing after balloon angioplasty. A larger UV-irradiation dose, which increases the amount of arterial wall damage, is equivalent to a higher 8-MOP dose at the same UVA dose [24,25]. Though, no significant negative correlation could be observed between 8-MOP serum level and induced intimal hyperplasia and arterial shrinkage. In fact, a trend ($R^2 = 0.319$, $P = 0.11$) was observed for an increased late loss for higher 8-MOP serum levels, which suggests that the UVA dose applied was sufficient or even too high. Surprisingly, preliminary studies in our laboratory suggest that 8-MOP activated by UVB irradiation reduces the enhanced effect of UVA activated 8-MOP on lumen loss [26], possibly due to the higher absorption of UVB by 8-MOP. Thus, the optimal UV irradiation dose as well as the opti-

mal wavelength for activation of 8-MOP for the inhibition of luminal narrowing after balloon dilation still has to be found out.

Photodynamic therapy for the prevention of luminal narrowing after arterial injury has been described since several years [17–19]. The rationale for this photo-activation study was the use of a photosensitizer, which gives rise to cytostatic instead of cytotoxic effects upon photo-activation, with a high excretion rate [27] to minimize the risk of systemic side effects. PUVA-therapy has been reported to inhibit intimal hyperplasia and luminal narrowing after 14 days of survival [14]. In contrast to the latter study and a number of PDT studies [17–20], our study does not support the combination of a photosensitizer and subsequent activation by light to be precautionary for the induction of intimal hyperplasia and luminal narrowing. This observation is in agreement with another study using pig coronary arteries [28].

A number of reasons for the apparent discrepancy in outcome with the above mentioned studies [14,17–20] can be hypothesized, for instance the above-mentioned different injury models and working mechanisms. However, the fact that those studies only focused on preventing intimal hyperplasia but not constrictive remodeling is also of interest. This notion is especially important with regard to the study by Moran et al., which showed a beneficial effect of PUVA therapy on angiographic luminal narrowing after 2 weeks [14]. However, their follow-up duration may have been too short to detect this phenomenon because remodeling is a late response after arterial injury [29]. The present study is the first to describe luminal narrowing after arterial wall injury with and without photosensitizer activation in terms of both intimal hyperplasia and constrictive remodeling. It appears from this study again that remodeling is an important parameter in luminal narrowing. Thus, the reported success of PDT or PUVA in preventing intimal hyperplasia in other studies may be overshadowed by the non-assessed remodeling of the arterial segment, rendering these regimes unsuccessful for the prevention of luminal narrowing after arterial wall injury. On the other hand, PDT or PUVA may prove to be an important therapeutic modality for the prevention of intimal hyperplasia after stenting [1,7].

Limitations of the Study

In dermatology, PUVA-therapy is a recurrent exposure treatment. In cardiology, recurrent intravascular exposures are too aggravating for the patient. For PUVA to be accepted as a therapy to prevent luminal narrowing after balloon dilation in cardiology, it is necessary to be able to administer the therapy during or shortly after an interventional procedure. Therefore, in this study, it was chosen to assess the effect after a single PUVA treatment. Although it is ambitious to extrapolate the results of animal studies to clinical applications, we think it is important to note that, in this study, no complications like occlusion of the lumen or aneurysm formation of the arterial wall were witnessed up to 4 weeks after PUVA therapy. Interestingly, at the single UVA and photosensitizer dose tested, enhanced arterial shrinkage was observed in this study. To elucidate an optimum dose for PUVA-therapy and the biological mechanism underlying the observed enhanced arterial remodeling, more experiments, including dose response studies, are necessary.

With respect to the mechanism of the effect of PUVA on arterial remodeling, two other limitations of this study are noteworthy. First, in situ dosimetry of the applied UVA dose would have been desirable, especially in the light of the limited penetration of UVA irradiation. However, preliminary studies in our laboratory have indicated that activation of the photosensitizer occurs even in the deep adventitial layer of the blood vessel (unpublished observation). Second, a balloon dilation only (without 8-MOP) group was absent in this study. However, the dark effects of 8-MOP are small compared to the biological effect of the photo-activated sensitizer [11,24].

Finally, before clinical application of PUVA-therapy during balloon dilation, additional experiments in an animal model which has more resemblance with human cardiophysiology than the rabbit, such as the atherosclerotic pig, are necessary.

Conclusions

PUVA-therapy at a radiant exposure of 1 J/cm^2 did not inhibit luminal narrowing after balloon angioplasty in the rabbit iliac artery under the specific conditions that were used in our study. PUVA-therapy even augmented luminal narrowing by enhanced constrictive arterial remodeling.

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